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Linkage Analysis in Three Families With Nonspecific X-Linked Mental Retardation

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Nonspecific X-linked mental retardation (XLMR) is a common disorder. The number of genes involved in this condition is not known, but it is estimated to be more than 10. We present a clinical and linkage study on 3 families with XLMR. All families were analyzed using highly polymorphic markers covering the X chromosome; screening for the fragile X mutation was negative. The first family (MRX 36) consisted of 1 female and 4 male patients in 3 generations and 7 healthy individuals. Considering the female as an expressing heterozygous carrier, a maximum LOD score of 3.41 was reached in region Xp21.2–Xp22.1. Considering her phenotype to be unknown, a LOD_{max} of 1.97 was reached in the same region. The second family consisted of 5 affected and 6 healthy males with mild to borderline mental retardation. Linkage analysis using an X-linked recessive model with full penetrance and no phenocopies excluded linkage over almost the entire X chromosome. Using alternative models, including an affecteds-only analysis, a LOD_{max} of 1.49 was found in region Xq24-28. The third family, consisting of 4 male patients with moderate mental retardation in 1 generation yielded a LOD_{max} of 0.9 in region Xp22.13–11.3. However, even in this small pedigree, exclusion mapping was able to exclude very large parts of the X chromosome and in this way identify a likely candidate region. © 1996 Wiley-Liss, Inc.

KEY WORDS: nonspecific X-linked mental retardation, linkage analysis, X chromosome

INTRODUCTION

The frequency of mental retardation, defined as subaverage intellectual functioning, is estimated to be 1–2% in the general population [Mercer, 1973; Jacobson and Janicki, 1983; Richardson et al. 1984]. Most epidemiological studies have indicated a higher frequency in males versus females, with an excess between 10 and 40% [Jastak et al., 1963; Jones, 1979; Baird and Sadovnick, 1985; McGuffin et al., 1994]. The male excess is probably due to the high frequency of X-linked traits causing mental retardation (XLMR). This frequency is estimated at 1/300 to 1/600 males [Glass, 1991; Kerr et al., 1991]. In a recent update, Neri et al. [1994] counted 127 X-linked conditions that involve mental retardation. Two groups of XLMR can be distinguished. The first group is comprised of X-linked conditions causing syndromal mental retardation; in this syndromic group, the fragile X syndrome is the most frequent single entity, with a prevalence of 2.5/10,000 in males [Turner et al., 1996]. The second group is comprised of families with nonspecific XLMR, i.e., nonsyndromal X-linked mental retardation. The overall population frequency is estimated to be at least as high as that of fragile X syndrome [Kerr et al., 1991]. To define regions that contain genes for nonspecific XLMR, linkage analysis is performed on these families. Because it is impossible to pool families by phenotype, linkage analysis results have to be interpreted separately in each family.

We present a clinical and linkage study of 3 families with nonspecific X-linked mental retardation.

Patients

The pedigrees of the 3 families involved in this study are shown in Figure 1.

Family H consists of 1 female and 4 male patients in 3 generations. Individuals II-1, II-4, and II-5 live with foster families as part of a project for mentally retarded persons and have nonsyndromal moderate retardation. Clinical and neurological examinations showed no abnormalities. Screening for metabolic disorders was negative. CAT scans of the brain were normal. Fragile X syndrome was excluded by cytogenetic and molecular techniques.

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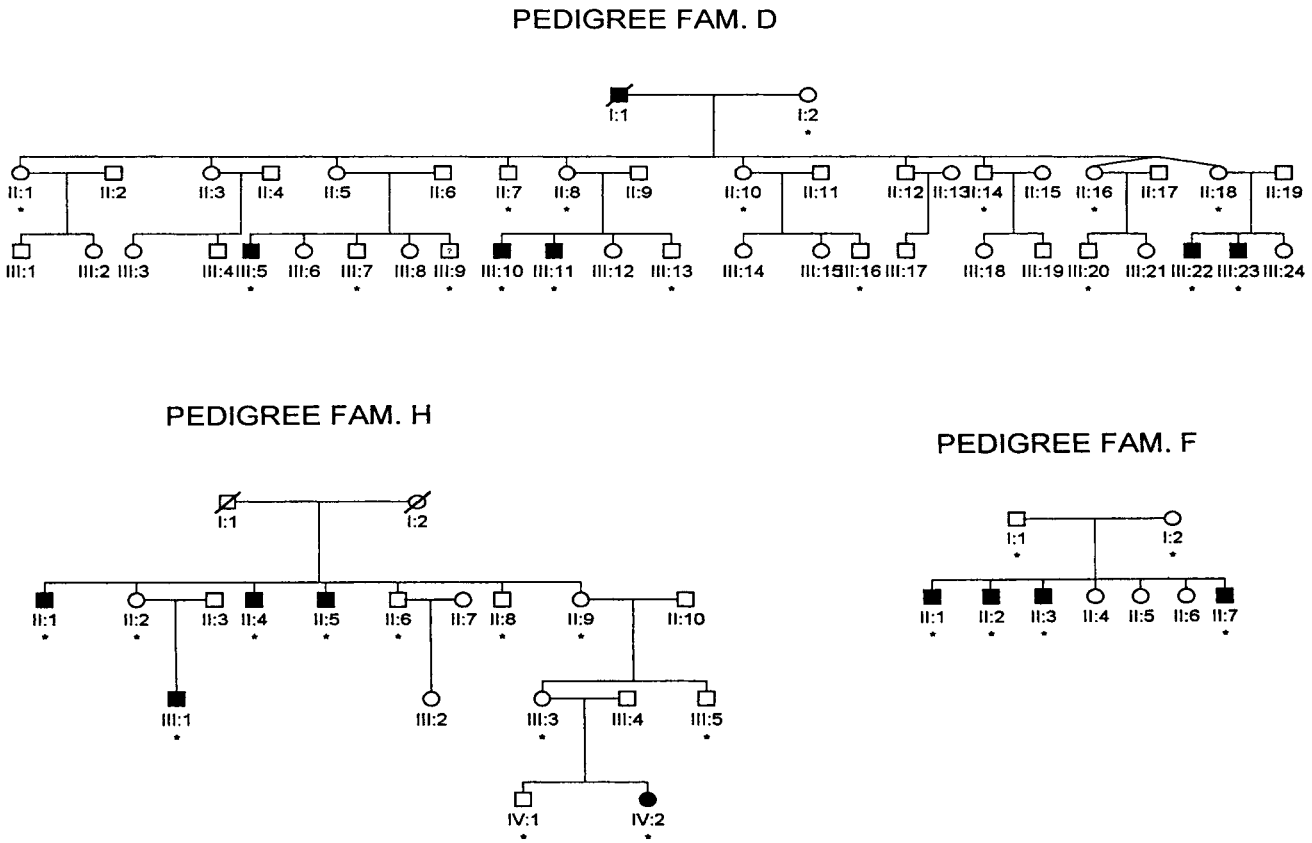

Fig. 1. Pedigrees of families F, H, and D. All individuals who were available for clinical examination and blood sampling are indicated by an asterisk.

Individual III-1 was enrolled in the special educational system for the mentally retarded. He lives with his mother and is mildly retarded. Clinical examination and screening for the fragile X syndrome were negative.

Individual IV-2 is a 20-month-old girl with marked delay in psychomotor development and relative microcephaly. From a clinical basis, it is not possible to determine whether she is retarded due to the same genetic factor as the males in the pedigree. Therefore, we performed linkage analysis under 2 models for the disease status of this girl: affected and unknown.

Individuals II-6, II-8, and III-5 followed the regular education system and show normal intelligence. There is a marked difference in intelligence level between the unaffected and the affected males.

Individual IV-1 is a 5-year-old boy with normal psychomotor development. Females II-2 and II-9 show an intelligence level in the low-to-normal range, possibly as a result of a heterozygous carrier status.

Family D consists of 5 affected and 6 healthy males in 2 generations. The clinical data in this family are summarized in Table I.

Patients show mild-to-borderline mental retardation, making it sometimes difficult to differentiate them from the healthy males in the pedigree. Patient III-5, at 30 years old, lives at his parental home. He was referred to a special education program for children with learning difficulties at age 10. He functions at a mildly retarded level. He has macrocephaly (HC = 63 cm, >p97).

Patients III-10 and III-11 were referred to a special education program for children with learning difficulties at the ages of 10 and 12 years, respectively, because of mental retardation and marked behavior problems. Formal IQ testing revealed IQs of 81 and 83, respectively. Patient III-10 also has relative macrocephaly (>p90).

Patients III-22 and III-23 were referred to a special education program for children with learning difficulties at the ages of 8 and 10 years, respectively, because of mild mental retardation and hyperactivity.

Individual III-9, who is now 14 years old, experiences major learning disability in the regular education system and functions on a borderline mentally retarded level. However, after a premature birth at 28 weeks, he developed a severe neonatal sepsis and was ventilated because of anoxemia. Therefore, we considered the cause of his condition to be unknown.

In all these patients, clinical examination showed no abnormalities, except for macrocephaly in individuals III-5 and III-10. Fragile X screening was negative. Several patients experience disturbances in motor skills, for which they receive prolonged physiotherapeutic treatment. All other individuals from the third generation and all individuals from the second generation fol-

TABLE I. Clinical Data in Family D

	III-5	III-7	III-9	III-10	III-11	III-13	III-16	III-20	III-22	III-23
Mental status	Mild MR	Normal	Borderline MR	Borderline MR	Borderline MR	Normal	Normal	Normal	Mild MR	Mild MR
Dropout from regular education system	Yes	No	No, but major learning difficulties	Yes	Yes	No, but major learning difficulties	No	No	Yes	Yes
Hyperactivity	No	No	No	No	No	No	No	No	Yes	Yes
Behavioral disturbances	No	No	No	No	Yes	No	No	No	Yes	Yes
Head circumference	>p97	p75–p90	p50	p90–p97	p75–p90	—	—	p75–p90	p75–p90	p50–75
Motoric disturbances	No	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Birth history	Born after 43 weeks gestation	Normal	Born at 28 weeks, severe neonatal anoxemia	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Disease status	Affected	Unaffected	Unknown	Affected	Affected	Unaffected	Unaffected	Unaffected	Affected	Affected
Penetrance (%)	100	60	—	100	100	30	60	30	100	100
Phenocopy rate	0.2	0.01	—	0.1	0.1	0.01	0.01	0.01	0.0001	0.0001

lowed the regular educational system and function at a normal mental level.

Individual I-1 died at the age of 67 of a cerebrovascular accident. He stayed for several months in a psychiatric hospital because of alcoholism and aggressive behavior. His intelligence level was described as low. Therefore, we hypothesize that the genetic defect was introduced into this pedigree by individual I-1.

Family F consists of 4 affected males in 1 generation. All 4 show moderate-to-mild mental retardation, with IQs ranging from 45 to 60. They all followed the education system for the mentally retarded and are now employed in a sheltered working environment. In all patients, clinical and neurological examination showed no abnormalities, and fragile X screening was negative. The 3 females in the second generation and both parents function on a normal mental level.

METHODS

DNA Analysis

Genomic DNA was extracted from venous blood according to standard procedures [Sambrook et al., 1989]. All genetic markers used in this analysis are microsatellites, except DXS52, which is a variable number of tandem repeats (VNTR). Polymerase chain reaction (PCR) amplifications of these polymorphic markers were performed with fluorescein-labeled locus-specific primer pairs (Genome Data Base). Marker genotypes of patients and relatives were determined by separating PCR products on a Pharmacia ALF automated sequencer (Pharmacia Biotech, Uppsala, Sweden). For marker DXS52, PCR products were separated on an ethidiumbromide-stained 2% agarose gel. Optimized PCR conditions, gel loading, and running conditions can be obtained from the authors.

Linkage Analysis

Two-point and multipoint LOD analyses were performed with Linkage Package 5.1 [Lathrop and Lalouel, 1984]. For males, penetrances were set at 0.0 and 1.0 for noncarriers and carriers of the mutant gene, respectively. For females, penetrances were set at 0.0 for noncarriers and heterozygous carriers and at 1.0 for homozygous carriers of the mutant gene. Phenocopy rate was set at 0.0. In family H, when analyzing model 2 (female IV-2 affected), penetrance for heterozygous carrier females was set at 0.2. Family D presented with mild-to-borderline mental retardation. For some individuals, it is difficult to determine whether or not they are affected. To cope with this problem, we assigned to each male individual in generation III a liability class, with specific penetrance and phenocopy values, reflecting the degree of uncertainty of diagnosis in this individual, based on the clinical data (Table I).

RESULTS

Family H

Twenty-four highly polymorphic markers covering the entire X chromosome were analyzed. The data were analyzed according to 2 models.

In the first model, it was assumed that the cause of the MR in individual IV-2 is unknown. Haplotype analysis showed complete linkage with markers DXS989, DXS1218, DXS992, DMD49, and DMD45 in the region Xp21.2–Xp22.2 (Fig. 2). Two-point (Table II) and multipoint (Fig. 3) LOD score analysis yielded a LOD_{max} of 1.97 at markers DXS989, DXS1218, DMD49, and DMD45. Flanking markers were DXS996 distally and DYSII proximally. Linkage can be excluded (LOD < -2) over the proximal part of the short arm and over the largest part of the long arm.

In the second model, the hypothesis was that individual IV-2 is retarded due to the same genetic factor as the males in the pedigree (disease status: affected). She inherited only a part of the candidate region under model 1. Haplotype analysis showed complete linkage with marker DXS1218, in the region Xp21, distal of the DMD gene (Fig. 2). LOD score analysis yielded a LOD_{max} of 3.31 at DXS1218 (Table II, Fig. 3). Flanking markers are now DXS989 and DXS992, leaving a candidate region of less than 5 cM (Xp21.2–22.13). Linkage can be excluded (LOD < -2) over the proximal part of the short arm and over the largest part of the long arm.

Family D

Twenty-seven highly polymorphic markers spread over the X chromosome were analyzed. Applying the regular model (see Methods), maximum 2-point LOD scores of 0.61 were found at 20 cM from markers DXS425, HPRT, and DXS1205 (data not shown). In the multipoint approach, linkage was excluded ($LOD_{max} < -2$) over the entire X chromosome, except for regions Xp11 and Xq21, where the LOD score is between -2 and -1 (data not shown).

With identical penetrance and phenocopy values including only the affected males in the analysis, a maximal 2-point LOD score of 1.49 was found at markers DXS424, DXS425, HPRT, DXS1205, and DXS1193 (Table III). In the multipoint linkage, a LOD_{max} of 1.49 was reached in the region distal from DXS424 (region Xq24–28) (Fig. 4). This is the maximal obtainable LOD

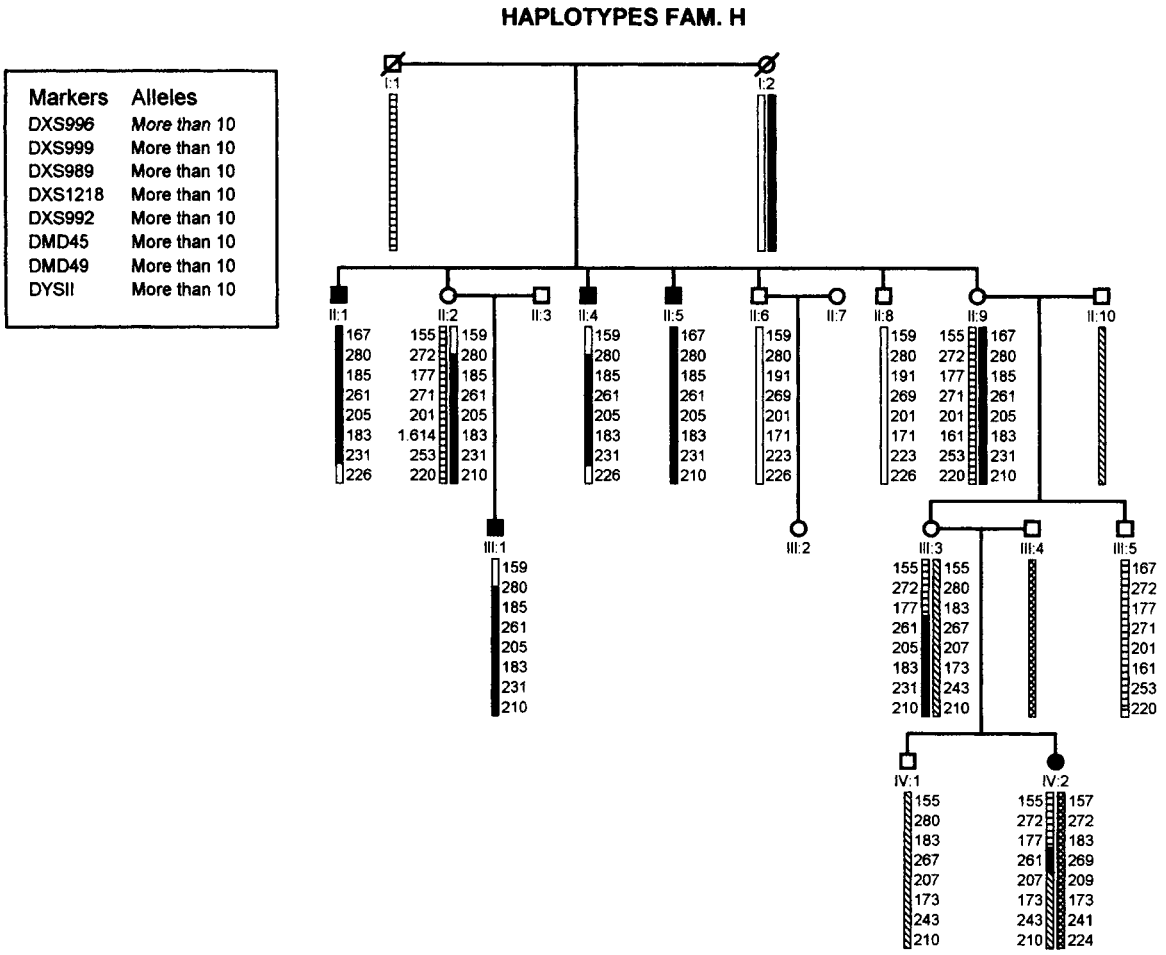

Fig. 2. Haplotype analysis in family H; the region between markers DXS999 and DYSII are shown (region Xp22.2–Xp21.2). All patients in generations II and III share the same haplotype. Individual IV-2 inherits only a small part of this region, which is between markers DXS989 and DXS992 (region Xp21.2–21.3).

TABLE II. Two-Point Linkage Analysis in Family H

Person IV-2 is included as an affected heterozygote female with penetrance at 0.2					Person IV-2 not included; heterozygote female penetrance at 0.0				
θ	0.0	0.01	0.05	0.1	θ	0.0	0.01	0.05	0.1
DXS996	−inf	−5.316	−2.628	−1.564	DXS996	−inf	−3.752	−1.749	−0.966
DXS999	−inf	−0.822	−0.176	0.056	DXS999	0.325	0.322	0.309	0.289
DXS989	−inf	0.966	1.486	1.552	DXS989	1.968	1.933	1.790	1.603
DXS1218	3.311	3.258	3.044	2.762	DXS1218	1.968	1.929	1.771	1.571
DXS992	2.709	2.661	2.464	2.205	DXS992	1.667	1.628	1.472	1.276
DMD-49	−inf	1.263	1.765	1.807	DMD-49	1.968	1.929	1.771	1.571
DMD-45	−inf	1.263	1.765	1.807	DMD-45	1.968	1.929	1.771	1.571
5′ DMD	−inf	−1.325	−0.070	0.343	5′ DMD	−inf	−2.349	−1.023	−0.513
DXS1068	−inf	−3.018	−1.065	−0.350	DXS1068	−inf	−2.061	−0.784	−0.330
DXS1003	−inf	−7.308	−3.884	−2.473	DXS1003	−inf	−4.052	−2.044	−1.256
DXS991	−inf	−8.710	−4.606	−2.917	DXS991	−inf	−4.052	−2.044	−1.254
DXS986	−inf	−7.011	−3.606	−2.218	DXS986	−inf	−4.052	−2.044	−1.254
DXS990	−inf	−9.303	−5.163	−3.427	DXS990	−inf	−6.039	−3.281	−2.128
DXS178	0.281	0.276	0.261	0.243	DXS178	0.017	0.017	0.015	0.013
DXS1001	−inf	−5.020	−2.349	−1.309	DXS1001	−inf	−3.751	−1.744	−0.958
DXS425	−inf	−1.622	−0.349	0.088	DXS425	−inf	−2.351	−1.032	−0.528
HPRT	−inf	−3.114	−1.732	−1.150	HPRT	0.149	0.149	0.147	0.143
DXS984	−inf	−4.523	−2.459	−1.598	DXS984	0.142	0.142	0.141	0.138
DXS1227	−inf	−3.617	−1.627	−0.861	DXS1227	−inf	−2.058	−0.771	−0.311
DXS292	−inf	−0.828	−0.181	0.052	DXS292	0.143	0.143	0.141	0.136
DXS998	−inf	−5.022	−2.333	−1.269	DXS998	−inf	−2.057	−0.765	−0.300
DXS1193	−inf	−1.118	−0.455	−0.199	DXS1193	0.149	0.147	0.138	0.127
DXS52	−inf	−5.020	−2.349	−1.309	DXS52	−inf	−0.061	0.513	0.653
F8	−inf	−5.316	−2.628	−1.564	F8	−inf	−0.061	0.513	0.653

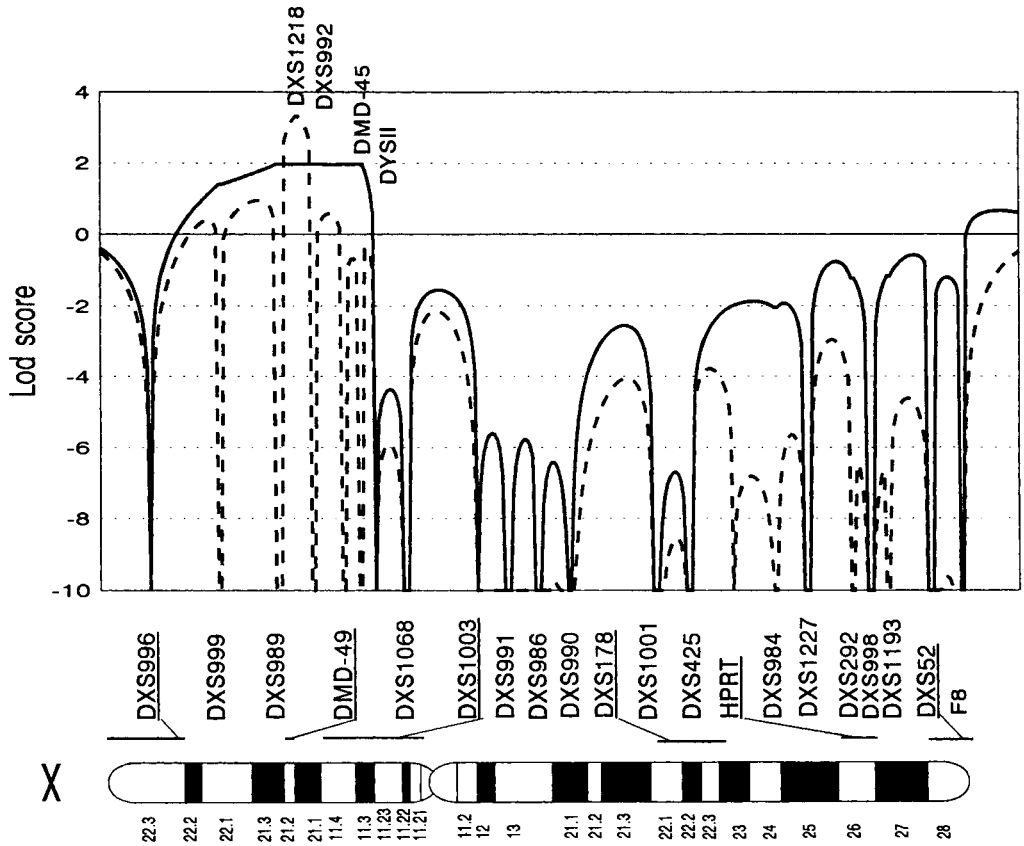

Fig. 3. Multipoint LOD analysis in family H according to 2 hypotheses: disease status of individual IV-2 affected (dashed line) and unknown (solid line).

TABLE III. Two-Point Linkage Analysis in Family D

Different liability classes for males in generation III					Affected individuals only				
θ	0.0	0.01	0.05	0.1	θ	0.0	0.01	0.05	0.1
DXS996	−1.999	−1.892	−1.488	−1.128	DXS996	−inf	−3.424	−2.071	−1.513
DXS999	−1.300	−1.225	−0.970	−0.732	DXS999	−inf	−3.112	−1.725	−1.140
DXS989	−4.227	−2.155	−1.249	−0.794	DXS989	−inf	−4.514	−2.446	−1.584
DXS992	−3.676	−1.649	−0.879	−0.526	DXS992	−inf	−2.695	−1.341	−0.797
DXS985	−3.676	−1.648	−0.878	−0.524	DXS985	−inf	−2.695	−1.341	−0.796
DMD49	−3.235	−1.214	−0.474	−0.159	DMD49	−inf	−2.521	−1.177	−0.645
DXS1068	−2.824	−0.834	−0.196	0.028	DXS1068	−inf	−1.118	−0.455	−0.200
DXS1003	−3.235	−1.214	−0.474	−0.159	DXS1003	−inf	−2.521	−1.177	−0.645
DXS991	−3.234	−1.212	−0.472	−0.157	DXS991	−inf	−2.521	−1.176	−0.643
AR	−3.620	−1.591	−0.817	−0.459	AR	−inf	−2.521	−1.177	−0.645
DXS986	0.404	0.401	0.385	0.362	DXS986	0.634	0.624	0.585	0.534
DXS990	0.404	0.401	0.385	0.362	DXS990	0.634	0.624	0.585	0.534
DXS178	−0.023	0.007	0.104	0.180	DXS178	−inf	−0.525	0.101	0.308
DXS424	1.219	1.203	1.137	1.047	DXS424	1.491	1.469	1.380	1.263
DXS1001	0.127	0.124	0.116	0.105	DXS1001	0.032	0.031	0.028	0.025
DXS425	1.219	1.203	1.137	1.047	DXS425	1.491	1.469	1.380	1.263
HPRT	1.219	1.203	1.137	1.047	HPRT	1.491	1.469	1.380	1.262
DXS984	0.127	0.126	0.123	0.117	DXS984	0.032	0.033	0.035	0.035
DXS1205	1.219	1.203	1.137	1.047	DXS1205	1.491	1.469	1.380	1.262
DXS1227	Not informative				DXS1227	Not informative			
DXS292	Not informative				DXS292	Not informative			
DXS1200	1.081	1.066	1.001	0.915	DXS1200	1.236	1.218	1.143	1.044
DXS998	−0.282	−0.270	−0.228	−0.182	DXS998	0.287	0.283	0.265	0.242
DXS1193	0.825	0.818	0.787	0.740	DXS1193	1.491	1.469	1.380	1.262
DXS52	−0.177	−0.170	−0.146	−0.119	DXS52	0.111	0.109	0.102	0.093
F8	Not informative				F8	Not informative			

score based on these 5 patients. Linkage was excluded over the short arm and the proximal part of the long arm ($LOD_{max} < -2$).

The haplotype shared by all patients in this region is the grandpaternal haplotype (Fig. 5), which is consistent with the clinical assumption that the grandfather carried the mutant gene (see Patients).

The third analysis assigned different liability classes to the males in the third generation, each with its own penetrance and phenocopy rate. The assignment of the liability classes was based on clinical criteria (see Table I). With this model, LOD score analysis yielded a LOD_{max} of 1.22 at DXS424, DXS425, HPRT, and DXS1205 (Table III, Fig. 4). Linkage was excluded over the short arm and the proximal part of the long arm.

Family F

Eighteen highly polymorphic markers spread over the X chromosome were analyzed. In the 2-point LOD score analysis, a LOD_{max} of 0.90 was found at DXS1068 (Xp11.4) (Table IV). This is the highest possible LOD score in this family. With the multipoint approach, a similar LOD_{max} was found at markers DMD49 and DXS1068. Flanking markers were DXS989 and DXS1003, limiting the likely candidate region to approximately 35 cM (Xp22.13–p11.3) (Fig. 6). The majority of the long arm of the X chromosome can be excluded except for region Xq21.3, where the LOD score climbs to −0.7.

DISCUSSION

In family H, 4 male patients and a 20-month-old girl with mental retardation were observed. By applying the most conservative model (disease status of individual IV-2: unknown), our data indicate that the XLMR gene in this family is located in region Xp21.1–Xp22.2, between markers DXS996 and DYSII. The number MRX36 was assigned to this family.

When individual IV-2 is considered to be affected, the candidate region becomes much smaller, whereas the LOD score increases significantly. When one considers individual IV-2 as an expressing XLMR female gene carrier, both II-9 and III-3 become nonexpressing heterozygous XLMR females. As a result, the meioses from I-2 to II-9, from II-9 to III-3 and III-5, and from III-3 to IV-1 and IV-2 all become potentially informative. Thus, by employing model 2, the total number of potentially informative meioses increases from 7 to 12. Accordingly, the maximal LOD score can increase by approximately 1.5. Conversely, the chromosomal region in common between patient IV-2 and her affected male relatives is surprisingly small. Indeed, her grandmother, II-9, still shares the whole haplotype with the affected males (Fig. 2), but individual III-3 shows a recombination between marker DXS989 and DXS1218, which are assumed to be separated by only 1 cM [Gyapay et al., 1994], limiting the distal border of the region to DXS989. Individual IV-2 herself shows a recombination between DXS1218 and DXS992, limiting the proximal border to DXS992. Finally, this leaves

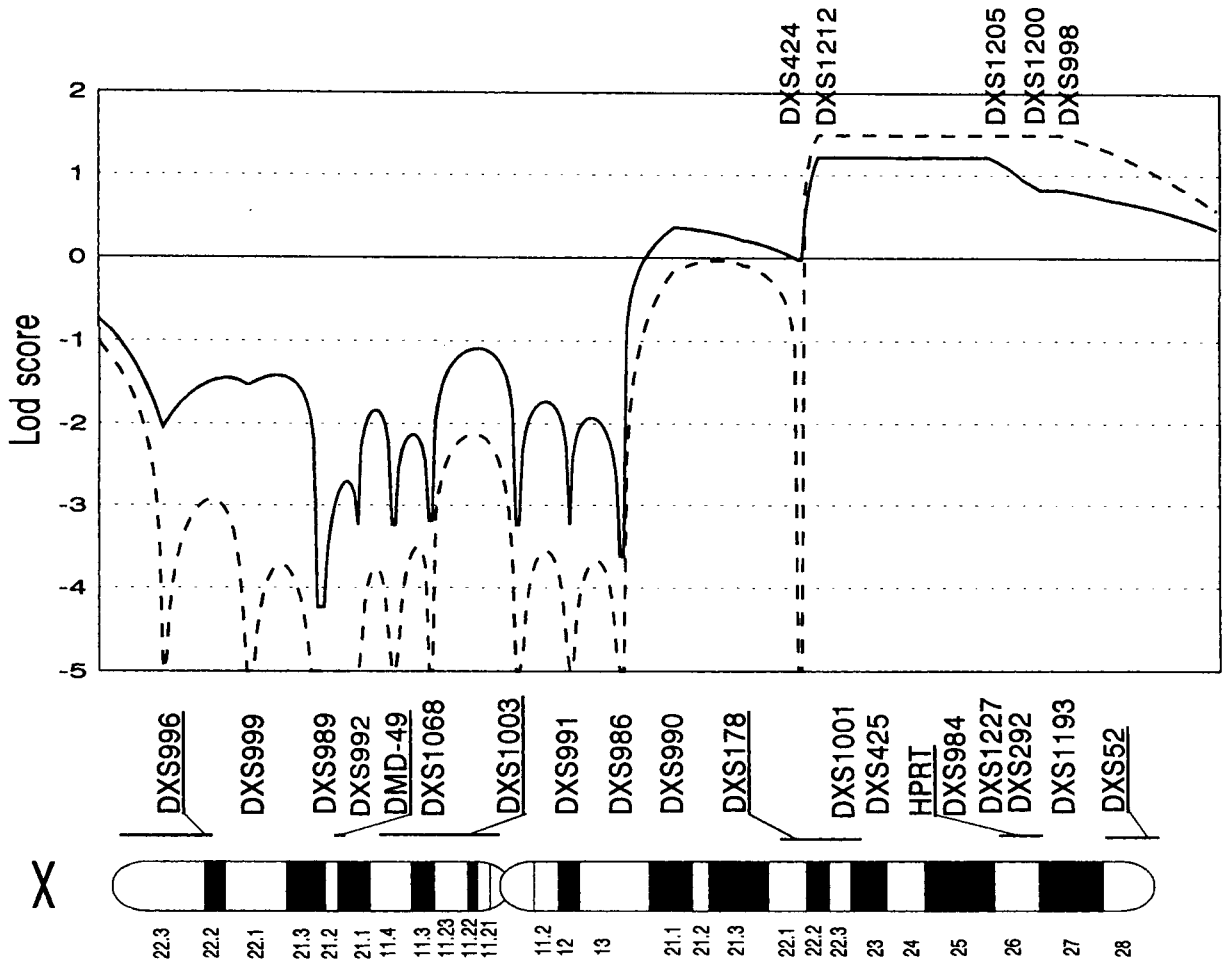

Fig. 4. Multipoint LOD analysis in family D with 2 models: one does not include the unaffected males in generation III (dashed line) and one assigns different liability classes (solid line).

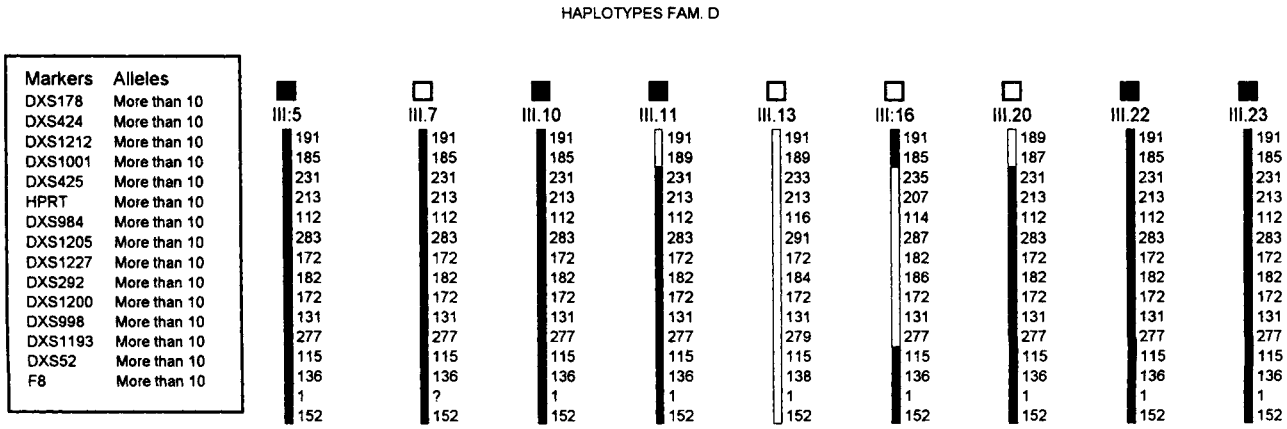

Fig. 5. Haplotype analysis in generation III of family D with the region distal from marker DXS178 (region Xq24–28). All affected patients share the same haplotype in this region; this is the grandpaternal haplotype (data not shown). Two unaffected individuals (III-13 and III-16) show a different haplotype. However, unaffected individuals III-7 and III- 20 have the same haplotype as the affected males.

TABLE IV. Two-Point Linkage Analysis in Family F

ϕ	0.0	0.01	0.05	0.1
DXS996	−inf	−1.109	−0.463	−0.228
DXS999	Not informative			
DXS989	−inf	−1.109	−0.463	−0.228
DMD-49	0.602	0.588	0.535	0.465
DXS1068	0.903	0.885	0.813	0.720
DXS1003	−inf	−1.109	−0.463	−0.228
DXS991	−inf	−2.804	−1.442	−0.887
DXS986	−inf	−1.109	−0.463	−0.228
DXS990	−inf	−1.109	−0.463	−0.228
DXS178	Not informative			
DXS1001	−inf	−2.804	−1.442	−0.887
DXS425	−inf	−1.109	−0.463	−0.228
HPRT	−inf	−1.109	−0.463	−0.228
DXS984	−inf	−1.109	−0.463	−0.228
DXS1227	−inf	−2.804	−1.442	−0.887
DXS292	−inf	−2.804	−1.442	−0.887
DXS1193	−inf	−2.804	−1.442	−0.887
DXS52	−inf	−1.109	−0.463	−0.228

a candidate region of only a few centiMorgans at approximately marker DXS1218. Very recently, a microdeletion in this region was found to cause nonspecific XLMR in an unrelated Belgian family (MRX34) [Raeymaekers et al., 1996]. In this pedigree, marker DXS1218 is located within the deleted region. Clearly, this marker amplifies normally in patients from family H. Based on the microdeletion in MRX34, it is reasonable to assume that there is a gene close to DXS1218 involved in intellectual development. If the disorder in family H is caused by a mutation close to DXS1218, it seems likely that the same gene is involved. Normal amplification with additional markers DXS1048, DXS1061, and DXS1202 [Gyapay et al., 1994], known to reside close to DXS1218, did not demonstrate the presence of a microdeletion in family H, although only a relative coarse marker coverage was used. Alternatively, the trait in family H might be caused by a point mutation. That anticipation might be a factor involved in the aggravation of the phenotype of IV-2 versus her affected male relatives is intriguing. In several other neuropsychiatric disorders, anticipation was described and found to be caused by the expansion of trinucleotide repeat sequences [Ross et al., 1993].

Family F has a pedigree with four males in one generation. The pedigree is too small to yield a significant proof of linkage; only an indication of a likely candidate region can be found (LOD_{max} = 0.9 in region Xp11.3–22.13). However, most of the long arm of the X chromosome can be excluded. Therefore, the relative

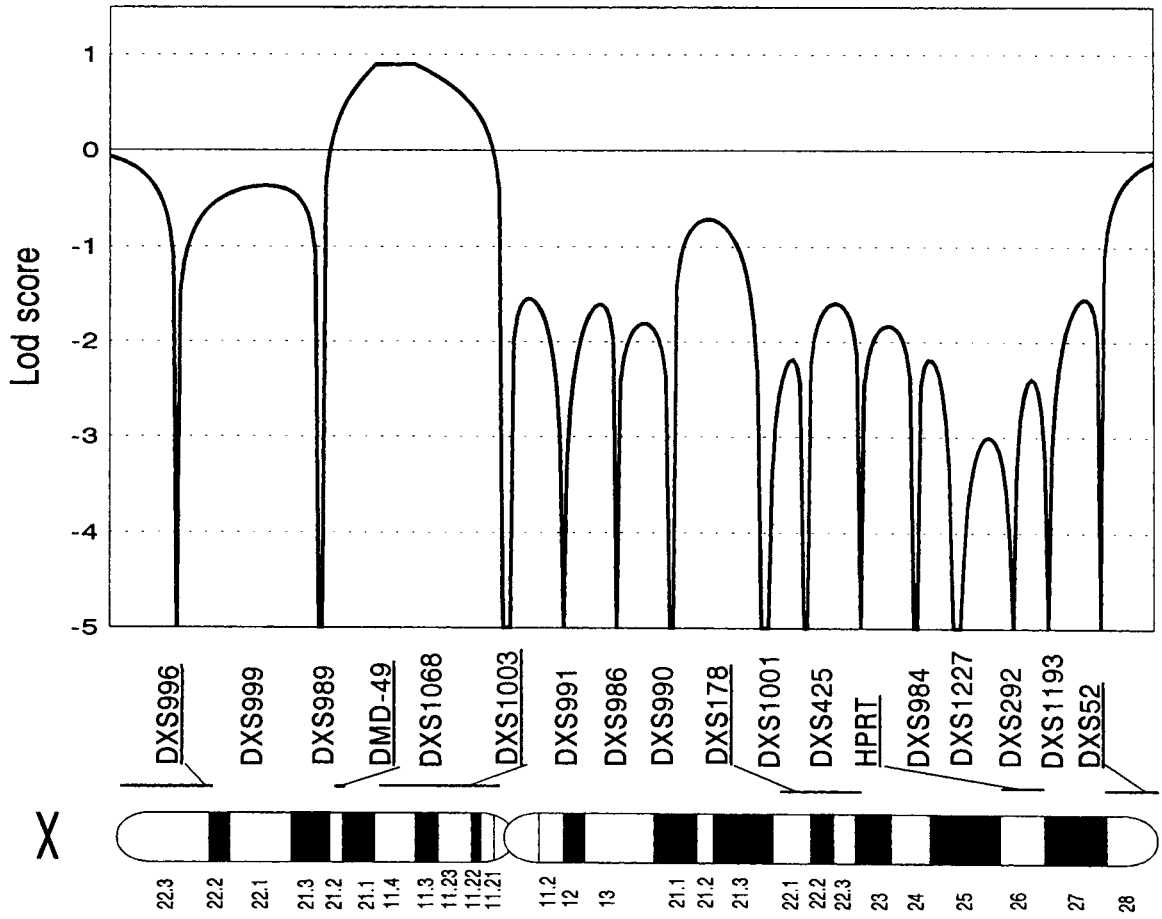

Fig. 6. Multipoint LOD analysis in family F.

likelihood that the mutant gene in this family is indeed located in Xp11.3–22.13 becomes much higher.

This proves that even in small pedigrees, where no significant LOD_{max} can be obtained, exclusion mapping is able to exclude very large parts of the X chromosome and indicate likely candidate regions. However, in such a small pedigree, an autosomal recessive transmission of the mutant gene is possible, although less probable than X-linked transmission.

The mutant genes in families H (regardless of the model used) and F map to overlapping regions in Xp. Genes for nonspecific X-linked mental retardation in several other families have been mapped to regions overlapping with this one: MRX2 [Arweiler et al., 1988], MRX9 [Willems et al., 1991], MRX10 [Kerr et al., 1992], MRX11 [Kerr et al., 1992], MRX12 [Kerr et al., 1992], MRX13 [Kerr et al., 1992], MRX14 [Gendrot et al., 1994], MRX18 [Gedeon et al., 1994], MRX19 [Donnelly et al., 1994], MRX21 [Kozak et al., 1993], MRX24 [Martinez et al., 1995], MRX29 [Hane et al., 1996], MRX31 [Partington et al., 1996], and MRX32 [Hane et al., 1996]. Additional studies will have to determine whether some of these families have a defect in the gene involved in the pedigrees described in this paper. Some metabolic disorders causing MR have been mapped in the same region: glycerol kinase deficiency, ornithine transcarbamylase (OTC) deficiency, and pyruvate dehydrogenase (DH) deficiency [Neri et al., 1994]. In addition, several XLMR syndromes have been linked to Xp11.3–22.13. However, there are no manifestations of any of these syndromes in families H and F.

The results in family D are indicative of the diagnostic problems that arise in families with mild-to-borderline mental retardation. Because the distinction between affected and unaffected is not always clear in these families, some "affected" individuals may not be gene carriers but individuals who are in the lower range of the normal IQ distribution. Some "unaffected" individuals may be XLMR carriers with reduced penetrance. The pedigree structure is highly suggestive for an X-linked genetic defect, but the analysis according to the strict X-linked recessive model (full penetrance, no phenocopies, and affected and healthy males included) excludes linkage with almost the entire X chromosome. To get around the problem of diagnostic uncertainty, an analysis including only the affected males was performed. In this analysis, Xq24–28 was found to be a likely candidate region (Fig. 4). However, by not including the unaffected relatives, the informativity is decreased and the LOD_{max} (= 1.49) is not significant, wherease linkage simulation indicated a possible LOD_{max} of approximately 2.5 under the strict X-linked recessive model. Although a LOD score of 1.5 is definitely not considered proof of linkage [Ott, 1991], relative odds for locating the mutant gene distal to DXS424 versus the region between DXS986 and DXS424 are at least 30:1 and are at least 5,000:1 to the pericentromeric region and the short arm. However, autosomal factors might be involved, but the pedigree structure is highly suggestive of X-linked inheritance.

A third analysis was performed to assign a different liability class to each male individual in the third generation based on clinical criteria. Again, linkage analysis points to region Xq24–27, but the significance level of 2.0 is not reached. In the haplotype analysis (Fig. 5), all affected males in the third generation share the same (grandpaternal) haplotype in this region. Two unaffecteds (III-13 and III-16) have a different haplotype. However, unaffected individuals III-7 and III-20 inherited the "affected" haplotype, and they might represent cases of reduced penetrance. In several families with nonspecific XLMR, the gene has been mapped to Xq24–28: MRX3 [Gedeon et al., 1991], MRX4 [Arweiler et al., 1988], MRX6 [Kondo et al., 1991], MRX25 [Nordstrom et al., 1992], MRX27 [Gedeon et al., 1996], and MRX35 [Gu et al., 1995]. Most of these MRX genes are located on Xq27–28, except for MRX27 and MRX35, which are located in Xq24–26. Furthermore, a paracentric inversion X(q21.2q24) has been found to cause mental retardation in males, possibly indicating an MRX gene on Xq24 [Abeliovich et al., 1995]. Several syndromic forms of XLMR have been mapped to Xq24–28. The most common is the fragile X syndrome, which was negative on cytogenetic and molecular screenings in this family. MASA syndrome, which was recently renamed "CRASH syndrome" [Willems et al., 1996], is caused by mutations in the L1CAM gene. However, no symptoms of this syndrome are consistently found in the affected members of family D. Macrocephaly (>p90), possibly associated with hydrocephalus, is found in 2 affected individuals but not in 3 other affected males. The other symptoms (adducted thumbs, shuffling gait, ataxia) are not found in this family.

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